V. *On the Protocerebrum of* Micropteryx (*Lepidoptera*).

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FOREWORD

More than four years ago I commenced to study the internal anatomy of *Micropteryx* (*Eriocephala*) in the hope that I might be able to throw some light on the question of its systematic position. As is well known, most entomologists regard it as a primitive Lepidopteron (Protolepidoptera), though there is really quite as good ground for regarding it as a Trichopteron.* I am now in a position to publish my results only in so far as they relate to a portion of the brain of that insect. This I do with a feeling

* *Vide*, however, Dr. Chapman's paper (Trans. Ent. Soc., 1916, pp. 310–4, pl. 81–93, (1917)), which raises *Micropteryx* to ordinal rank (order *Zeugoptera*).

that some apology should be made, because I do not at any rate describe the whole brain : it is owing to the war and pressure of other work that there is no likelihood of my being able to carry my investigations further. This paper is however complete in itself, and is not of the nature of a preliminary note. From the point of view of comparative anatomy, the Protocerebrum, with which alone this paper deals, is by far the most important part of the insect brain, not only on account of its complicated structure, but also because of certain questions relating to the homologies of some of its parts. Such questions of homology do not at present arise in connection with the Deutocerebrum or Tritocerebrum, or ventral brain. My hope that my investigations would throw light on the systematic position of *Micropteryx* will not be fulfilled until we can compare the brain of this insect with a number of other Lepidopterous and Trichopterous brains, after they have been fully and properly investigated.

I have made some attempt to render this paper useful also as an introduction to the study of the brain of insects; this I think is justified, because it is the first paper on the subject published in Great Britain since 1878 (Newton), and I know by bitter experience how exceedingly difficult it is to obtain a clear knowledge of the subject from a variety of papers written by many men in many languages at different dates. My task has been rendered difficult by the small size of *Micropteryx*, which is, I believe, the smallest insect of which the brain has been investigated in any detail.

I take this opportunity of acknowledging how much I am indebted to many friends in the University's Zoological Laboratory at Cambridge, particularly to Mr. F. Balfour Browne for constant criticism and much good advice, and for reading through the whole paper before it was published; also to Mr. L. A. Borradaile for helping me with the theory of the segmentation of the head of the Arthropoda; also to Dr. D. Keilin of the University of Paris, and of the Quick Laboratory and Magdalene College, Cambridge, for putting at my disposal his deep knowledge of fixing and staining. Canon W. Brocas Waters gave me a room to work in, while I was stationed in Bury St. Edmunds on military duties.

I must also acknowledge my indebtedness to Dr. K. F. Kühnle of Stuttgart for his paper on the brain of the

Earwig and other types (see Bibliography), which gives a full review of insect neurology up to 1913, together with a bibliography and a table of the terminology of the insect brain, which has materially lightened my labours. This paper is by far the most important contribution which has yet been made to insect neurology.

All my material has been collected in the neighbourhood of Cambridge, and I have worked entirely with *Micropteryx* (*Eriocephala*)* *calthella* (Linn.), and not with any other species.

INTRODUCTION.

The anterior part of the central nervous system of insects consists of a supraœsophageal portion, which is the brain in the narrow sense of the word. From this the circumœsophageal commissures pass round the œsophagus to the ventrocerebrum or subœsophageal portion of the brain. Most authors include this also in the brain of the insect. The supraœsophageal ganglion, or brain in the narrow sense of the word, was found by Viallanes to be composed of three paired elements or neuromeres, which he believed to be segmental; these give rise to the parts of the brain to which he gave the names Protocerebrum, Deuterocerebrum (or Deutocerebrum) and Tritocerebrum. We now know that the Protocerebrum is not a segmental ganglion; and it will be convenient at this point to give a short summary of the results obtained by those who have studied the subject of the segmentation of the head of the Arthropoda, and the homologies of the various appendages throughout the class from the point of view of comparative embryology. The whole matter is one of great difficulty, and has been neglected by insect neurologists; as, however, it is a matter which bears directly upon the subject of this paper I give this summary of our knowledge in so far as it affects the insect protocerebrum.

* The subject of this paper belongs to the true genus *Micropteryx* Hübner. This genus has been referred to in some writings, *e.g.* Meyrick's Handbook, the Cambridge Natural History, etc., under its synonym *Eriocephala* Curtis: while the leaf-mining genus erroneously called "*Micropteryx*" in certain of the same works should be known as *Eriocrania* Zeller.

Eriocephala Curtis = *Micropteryx* Hübner.

"*Micropteryx*" auctt. (nec Hübner) = *Eriocrania* Zeller.

See Tutt, Brit. Lep., I, pp. 129–137 (1899), and Staudinger-Rebel, Cat., II, pp. 246–8 (1901).

The brain of the Arthropoda in its fullest development, that is to say as exemplified in the brain of the embryo of *Scolopendra* (Heymons), consists of the following parts: an archicerebrum, which is median, unpaired and preoral: three lobes on each side, the syncerebral lobes, the outer two of which arise from a common rudiment; these also are preoral, and together with the archicerebrum form the syncerebrum: the preantennary ganglion, or protocerebrum, which is the ganglion of the first somite, or true segment; we believe that this was primitively postoral, but it is preoral in all living Arthropoda: the deuterocerebrum or antennary ganglion, and the tritocerebrum or premandibular ganglion, which correspond respectively to the second and third somites. It may be said at once that the deuterocerebrum and the tritocerebrum correspond in Heymons' nomenclature to the organs which I shall subsequently describe under those names. This is not, however, the case with the protocerebrum, for that word has been used in a great variety of senses. In the development of the insect head that part of the central nervous system which entomologists generally call the protocerebrum (Viallanes) is developed from the archicerebrum and the syncerebral lobes: we do not yet know which parts of the insect brain correspond to which of these structures, except that the outer syncerebral lobe gives rise to the optic lobe, and Haller suggests that the mushroom body is formed from the archicerebrum. The preantennary ganglion or protocerebrum of Heymons is not found at all in the insect head, and is to be carefully distinguished from that part of the brain which is commonly called by that name. The synonymy is further complicated because the preantennary ganglion or protocerebrum of Heymons is the precerebrum of Verhoeff, and the word "protocerebrum" has been used in yet a third sense to denote the procerebrum of Heymons, that is the syncerebrum and preantennary ganglion (protocerebrum) together. The word protocephalum has been used by Holste, and perhaps by others, to denote that part of the brain which is dorsal to the gut in the insects: *i. e.* the syncerebrum of Heymons (the protocerebrum of insect neurologists since the time of Viallanes), with the deuterocerebrum, and the tritocerebrum.

I shall continue to use the word protocerebrum in the sense in which neurologists from the time of Viallanes have always employed it, though I should be glad to avoid a

word to which so many meanings have been assigned. By it I mean a mass of nerve tissue arising from that preoral part of the embryo which is not segmented and which bears no appendages. It is the nervous element corresponding to the acron of some embryologists, and it is not the serial homologue of the deuterocerebrum and tritocerebrum (mesocerebrum and metacerebrum of some writers on the segmentation of the Arthropoda). The protocerebrum of insects is, in fact, the syncerebrum of Heymons, unless it contains some element not yet differentiated as belonging to the preantennary ganglion.

In this paper I propose as I have said to deal solely with the protocerebrum. I give, however, the following brief summary of the function and connections of the other two supraœsophageal ganglia. The deuterocerebrum is the ganglion of the antenna, to which it gives motor and sensory nerves. The pair of ganglia forming the deuterocerebrum are united across the middle line above the œsophagus, and lie before and below the protocerebral lobes. They are the antennary or olfactory lobes of some authors. The deutero-cerebrum gives rise to the paired sympathetic system, which lies upon the lateral wall of the œsophagus on each side; this consists of two pairs of small ganglia with nerves which connect them to each other, and, as is known in some insects, to the median or tritocerebral sympathetic system. The pair of ganglia composing the tritocerebrum lie on each side of the anterior part of the œsophagus and are generally fused above to the rest of the supraœsophageal brain. The lower part of the tritocerebrum is the circumœsophageal connective or commissure. This ganglion supplies the labrum, but has no paired appendage connected with it in the insects; a band of fibres, the tritocerebral bridge, passes across from one side to the other beneath the œsophagus. The tritocerebrum also gives rise to a pair of fine nerves which run forwards and inwards to the frontal ganglion, which lies upon the upper surface of the anterior part of the œsophagus. This is the largest ganglion of the sympathetic system: from it a fine nerve runs forwards and another backwards. This latter, the *nervus recurrens* of some authors, connects the frontal ganglion with a short chain of ganglia lying on the upper surface of the œsophagus, and from this unpaired sympathetic system the stomodaeum is innervated.

The subœsophageal ganglia or nerve masses will not

again concern us. They are formed by the fusion of four segmental ganglia, the mandibular, the intercalary, the maxillary and the labial. The intercalary ganglion has hardly been noticed by insect neurologists; the corresponding appendage is the maxillula, which is vestigial or absent in adult insects; the ganglion is accordingly ill-developed or absent. The other three ganglia are mainly if not entirely motor and sensory centres to their respective appendages.

All the nerve centres of insects consist of the following layers.* They are bounded externally by a neurilemma, which is a thin syncytial structure. Within this, and lying loosely in a quantity of fluid, are the nerve cells, or ganglion cells. The processes of these, the axons, pass inwards to form the innermost part of the centre; here they unite in very large numbers to form the tissue known as axonic substance (or fibrillar material), which consists of innumerable axons and their twigs bound together by a varying amount of neuroglia. Of this axonic substance two types may be distinguished; the first is that which is called Punktsubstanz, or Marksubstanz, or neurospongium; its composition was first accurately explained by Viallanes. Until his day it had been known as a tangled web, but in it he distinguished very fine axis cylinders running in all directions, and their twigs, and also the neuroglia. In the second type of fibrillar substance, the Fasersubstanz, the axons run in bundles and form well-defined tracts in which there is little or no neuroglia. The distinction between these two types must not be insisted upon, for every degree of intergradation may be found; even in the most indisputable Punktsubstanz small tracts of fibres may generally be detected. Physiologically again the difference is one of degree, though Fasersubstanz is mainly a tissue of conduction, Punktsubstanz one of association, that is to say one in which impulses pass from one neuron to another.

Two parts of the brain may be connected either by fusion of their component Punktsubstanz (*Verschmelzungen, soudures*), or by definite tracts of Fasersubstanz (*Faserverbindungen*). This distinction, again, has only a relative value.

Before we pass to examine the structure of the various

* The general relation of neurilemma, cells and axonic substance is shown on Plate X.

parts of the protocerebrum it should be realised that this portion of the brain is not merely a complex but also an entity. We might compare it physiologically to the cerebrum of a vertebrate. Both are known to be the highest or governing centres of the organism; both possess a solidarity or unity of action; both consist also of parts, each of which in its turn is not only an anatomical, but also a physiological entity. We are quite justified in regarding the protocerebrum of an insect as the headquarters from which are directed all those complicated reactions and instincts of the organism which give to its activities what at any rate appear to be purpose, and adaptation to the surroundings. There is too great a tendency to lay emphasis on the potentialities for independent action which are undoubtedly possessed by the lower nerve centres of the insect. In all but the very lowest insects there is a marked degree of specialisation in the structure and function of the protocerebrum, and this fact of its solidarity should not be allowed to pass from the mind while we study in detail the structure and perhaps something of the function of its parts.

THE PROTOCEREBRUM OF MICROPTERYX.

I. The Neurilemma and Ganglion Cells.

The whole brain of *Micropteryx*, that is to say the axonic parts and the cells, is included in a limiting membrane or Neurilemma (Plate X). This is a very fine sheet of substance which stains well with the acid stains. It is certainly a syncytium in *Micropteryx*, and probably in all insects. In places where no ganglion cells intervene between the neurilemma and the axonic part of the brain the two are closely applied to each other, and the neurilemma can hardly be distinguished, though in material fixed in the picro-chlor-acetic mixture it can generally be seen. Occasionally the nuclei of the neurilemma can be seen even when the layer itself is indistinguishable. The neurilemma is somewhat thickened mid-dorsally, partly owing to the fact that a number of tracheal tubes (Plate X) lie in it in this position, partly owing to the presence of a number of the nuclei, the cells corresponding to which have fused to form the syncytium of which the neurilemma consists. These nuclei are elongate and smaller than those of

"normal" ganglion cells. They stain deeply with hæmatoxylin. The neurilemma is continuous over the whole brain dorsally and ventrally and also over the optic lobes; it is continued downwards to cover the ventral parts of the central nervous system.

The Ganglion Cells.—These are spread over the whole anterior and superior parts of the brain, in a layer which reaches its greatest thickness mid-dorsally. The layer is discontinuous or absent beneath the protocerebrum. On the upper side of the brain the cells may be as many as 15 cells deep, particularly near the middle line between the ocelli. Various types of ganglion cell must be distinguished. They all possess a spherical nucleus and a very small quantity of cytoplasm. The normal cells (*g. c.*) cover the protocerebrum above, before and behind. Cells of this type, which is much the most abundant, are either motor or else cells of connecting-fibres (Kenyon). The cells of the mushroom body (*mb. c.*) are found as a rounded mass of cells lying just over the head of that organ. Their nuclei stain heavily with hæmatoxylin; they are also smaller than the normal cells. The fibres from these cells pass into the mushroom body. The distinction in size between these cells and those of the normal type is not very great; it can be best observed in material fixed in Gilson's fluid. The cells of the optic lobes (*o. c.*) are still smaller than those of the mushroom body; and their nuclei are absolutely spherical and stain very heavily and completely. No structure within the nucleus can be detected in ordinary sections and this gives to the masses of cells a very characteristic appearance. The cytoplasm, per contra, is scarcely stained at all. These cells form a deep coating which completely envelopes the three optic ganglia; this layer is less deep above than below. In *Micropteryx* giant cells (*gi. c.*) are found in small numbers round the base of the mushroom body just where it passes into the protocerebral lobes (fig. 12, etc.). These cells are few in number, hardly more than a score on each side. Their nuclei are spherical and about four times as large as those of normal cells; there is a considerable quantity of cytoplasm, which can be stained with eosin; this distinguishes it at once from the cytoplasm of the other types of ganglion cell. The nucleolus is generally clearly seen. Haller states that these cells are mainly, but not entirely, concerned with conduction across the middle line, and that their axons

pass to the opposite side of the brain, to the antennary lobe or head of the mushroom body, or even into the optic lobe. Inside the brain are found small cells, lying singly or in groups (Plate X). Kühnle refers to them as neuroglia cells (*ng.*) and doubtless he is correct in so doing. They are found particularly in the space surrounding the central body and in the interval between the two capsules of that organ; there are also a few on the surface of the stem of the mushroom body and in other places (Pl. X). The nucleus of a neuroglia cell is pyriform or elongate and stains deeply, and its outline is generally irregular. The nucleus is smaller than that of a "normal" ganglion cell.

The axons from the ganglion cells enter the axonic part of the brain vertically; and they are generally united into small bundles at their point of entrance. It is to this that Kühnle gives the name *Einströmmung*.

Spherical black granules occur among the cells in material fixed in osmic acid, or any mixture containing osmic acid. These granules are not found in material fixed in any other fluid, and I regard them as unsaturated fat. They are found among the ganglion cells and are quite definitely extracellular.

A note on technique is given at the end of the paper; suffice it to say here the cells may be studied in material fixed in the picro-chlor-acetic fluid, but that some specimens may with advantage be fixed in Bouin's or Gilson's fluids, especially for the study of the different types of cell.

Tracheation. — In the brains of most insects fine tracheal trunks can be detected ramifying in the fibrillar part of the organ. This is not the case in *Micropteryx*, perhaps because of the extremely small size of the whole insect: so far as I can discover there are no tracheae at all, either in the ganglion cell layer or the axonic fibrillar part of the brain. There is a considerable collection of tracheal trunks (*tr.*) in the neurilemma which lies over the mid-dorsal part of the brain (Plate X), and it is at least possible that it is the function of these trunks, which are large and numerous, to oxygenate the brain by diffusion through the fluid which lies beneath the neurilemma.

II. The Protocerebral Lobes.

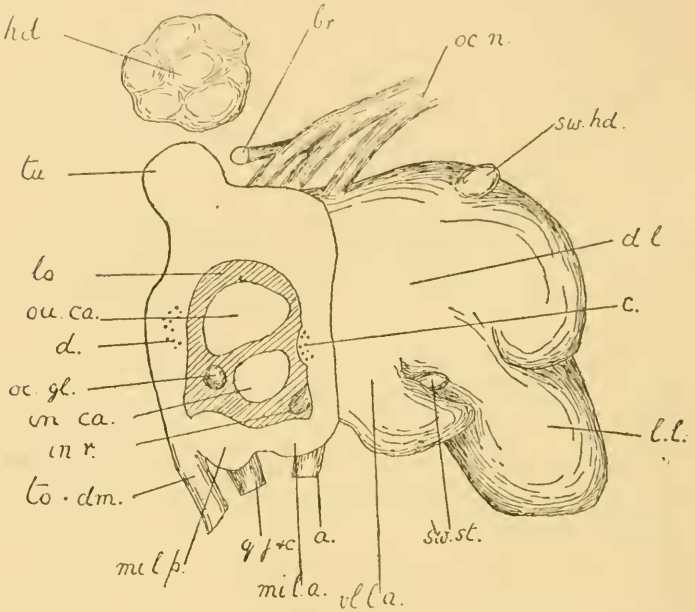
The protocerebrum of insects is generally described as consisting of the protocerebral lobes and the various structures such as the mushroom body, the central body,

the bridge, the ocellary glomerulus, and the optic lobes. The term "protocerebral lobes" is a comprehensive term for the great mass of the protocerebrum in or upon which the other structures lie. The word "lobes" is perhaps unfortunate, but its use in all papers from an early date to the present day renders it a classical term. In all insects the protocerebral lobes (*pc. l.*) form by far the greater part of the brain; they are bilaterally symmetrical about the middle line, but they are not divided from one another by a raphe. Kühnle describes their fusion across the middle line (*Verschmelzung*), "above," "below," etc. This is perfectly accurate, but it would give a clearer impression to say that the two lobes are united over their whole extent at the middle line, except that in the centre a space (*la loge*, Viallanes) is left in which lie the central body and ocellary glomeruli, and the inner root of the mushroom body.

The union of the two sides in *Micropteryx* is complete, but much less definite posterodorsally. Only a very vague web of fibres covers the central body in this region. It would, for instance, be possible for a micro-organism to swim down from the fluid in which the ganglion cells lie through this web into the space surrounding the central body. The protocerebral lobes together form a rounded mass, with its longest axis in the transverse direction. The mass is flattened above, and prolonged downwards to fuse with the deuterocerebrum and the tritocerebrum. The dividing line between the deutero- and trito-cerebrum cannot be accurately determined. In the embryo they lie behind one another. In most adult insects, and *Micropteryx* is no exception to the rule, the deuterocerebrum is pushed forwards, and the tritocerebrum fuses directly with the protocerebrum, at any rate by a small part of its posterior surface. From the lateral side of the protocerebrum the optic nerve is given off. This connects the protocerebrum to the optic lobes (medullary masses of the eye).

The relations of the protocerebral lobes are as follows (text fig. 1, p. 122): mid-dorsally lie the rounded heads of the mushroom bodies, and between them the bridge. Slightly in front of this the ocellary nerve is seen, disposed in a transverse plane with a slight inclination downwards and backwards. Various organs lie within the protocerebral lobes in a space full of fluid which has been called *la loge* by

Viallanes. These organs are the central body and the ocellary glomerulus, and the stem and inner root of the mushroom body; the forward and backward roots are also buried in the protocerebral lobes, but they are not definitely separated from the surrounding parts by a free space. It may be mentioned here that the mushroom body system is completely buried in the protocerebrum except at three points. The parts which project are the head, the lower end of the stem, and the tip of the forward root (see pp. 125 *sqq.*).



Text figure 1.—General relationships of the parts of the protocerebrum. The organ is divided at the middle line and the left side is shown in the figure, viewed from in front. The cut surface shows the loge of Viallanes and the organs within it. No cells are shown; the whole organ as drawn here consists of axonic substance. *a*, *c*, *d*, *j*, *q*, refer to tracts of fibres (see p. 136). *br.* bridge. *d. l.* dorsal protocerebral lobe. *hd.* head of mushroom body. *in. ca.* inner capsule of central body. *in. r.* inner root of mushroom body. *l. l.* lateral lobes of protocerebrum. *lo.* la loge (Viallanes). *mi. l. a.* and *mi. l. p.* anterior and posterior parts of middle lobe of protocerebrum. *oc. gl.* ocellary glomerulus. *oc. n.* ocellary nerve. *ou. ca.* outer capsule of central body. *sw. hd.* swollen head of ascending branch of mushroom body. *sw. st.* swollen foot of stem of mushroom body. *to dm.* tracts passing from protocerebrum to deuterocerebrum. *tu.* tumulus. *vl. l. a.* anterior part of ventrolateral lobe.

Special names have been assigned to various parts of the protocerebrum (refer to Pl. VII–IX). Thus dorsally there is the dorsal lobe (*Hauptlappe*), below this the ventrolateral lobe or *Nebenlappe*, and midventrally the middle lobe (*Mittelstück*). These parts may all be distinguished in *Micropteryx*, and perhaps the mere shape of the lobes merits description. The dorsal lobe (*d. l.*) is the widest part of the whole brain. In front its superior surface is flat; further back there is a specialised rounded projection in the middle line, to which I give the name Tumulus (*tu.*). This lies between the heads of the two mushroom bodies, and consists of a very tight homogeneous web of axonic substance more densely compacted than any other part of the brain. The portions of the dorsal lobe which lie around and beneath it are of an extremely loose consistency (see Pl. X).

A large lateral lobe (*l. l.*) is present on each side.* Its relations are shown in figs. 10, 12, 13.

The ventrolateral lobes (*Nebenlappe*) consist of two very definite parts placed one in front of the other. The anterior part of the ventrolateral lobe (*v. l. l. a.*) appears as a swelling below the anterior extremity of the stem of the mushroom body; in the region beneath the inner root of the mushroom body the lobe is insignificant; and behind this its posterior part (*v. l. l. p.*) appears as a large round lobe above the exit of the motor antennary nerve from the deuterocerebrum.

The middle lobe (*mi. l.*) of the protocerebrum lies between the two ventrolateral lobes. In most insects it consists of a single body, shaped like an hour-glass, and lying transversely between the inner roots. In *Micropteryx* we can distinguish an anterior and a posterior part of the lobe. The anterior portion (*mi. l. a.*) is of the shape of an hour-glass, and lies, as it should, between the ends of the inner roots; it is connected with the anterior part of the ventrolateral lobe on the same side by a tract of nerve fibres (tract *h*). Behind it there is a transverse bar of axonic tissue, placed below the ocellary glomeruli and above the various bands which connect the two antennary lobes or deuterocerebra; this is the posterior part (*mi. l. p.*) of the middle lobe; to the antennary lobe and also to the protocerebrum above it this middle lobe is united by well-marked tracts of nerve fibres (tracts *j* and *k*).

* This is not *le lobe latéral du protocérébron moyen* of Viallanes, which is the ventrolateral lobe.

Histologically the whole of the protocerebral lobes are very uniform in structure, and consist of Punktsubstanz of a moderate degree of density. The tumulus, however, is very much closer in structure, and the parts of the protocerebral lobes immediately below and around it are very loosely formed. The lobes are penetrated in all directions by bundles of axons (Fasersubstanz), some of which are enumerated below.

III. The Mushroom Body.

(Stalked body—Packard. Pilz—Kühnle, etc. Gestielte Körper—Leydig. Les Corps Pédonculés—Dujardin.)

Before I describe the mushroom body of *Micropteryx* it may not be out of place to state that the organ consists typically of a cup-shaped or globular head (*calice, Pilzhut, Becher, lobe à convolutions*) supported by a stem (*Stamm, tige, cauliculus, pedunculus*) which divides below into a number of roots or branches. The word *Stiel* is used by Kühnle to denote the stem and roots together. An early worker, Newton, described the brain of the cockroach. In this insect the head and stem of the mushroom body are double, and Newton named the two stems the cauliculus and pedunculus, respectively. A small number of insects have their mushroom body formed on a simpler plan, with only one head and one stem. It is better, therefore, not to use the terms cauliculus and pedunculus, which are responsible for the notion that two supports of the mushroom body are to be looked for in the typical insect brain. As will be seen later the homologies of the roots of the organ are very obscure.

This exceedingly simplified account of the least complex type of mushroom body may serve to remind the reader of the essential characters of that organ. A full account of that of *Forficula*, together with a painstaking summary of previous work, is given by Kühnle. This is valuable, but as I shall explain later I believe that Kühnle has made a fundamental mistake in homology. The summary in Packard's text book is out of date and most difficult to understand.

At first sight it appears that the mushroom body of *Micropteryx* is formed on a plan not altogether identical with that found in other insects. This is not very surprising, for nothing is yet known of the brains of the *Lepidoptera* or *Trichoptera*. I hope, however, to show that the diffi-

culties are rather apparent than real, and that *Micropteryx* is really one of those organisms from the study of which we may draw valuable inference as to the comparative anatomy of the insect brain. It is always a most difficult thing to form a picture of the mushroom body of an insect when that organ is described by another worker. This is due, in part at least, to the complexity of the organs which lie in all three planes of space. I shall endeavour to make my meaning clear by giving several sketches of a mental reconstruction of the organ in question. Its structure in *Micropteryx* is comparatively simple, and I have not found it necessary to make a wax-plate model. I have, of course, most carefully examined sections in all three planes of space (text figs. 2, 3, pp. 128–9).

The head of the mushroom body of *Micropteryx* is a single globular mass of axonic tissue, and belongs to the *Höcker* type of Kühnle. It appears that a similar structure was described by Flögel in certain moths; but it is never easy to understand his descriptions, for he was much hampered by the defective methods of his time.

The head of the mushroom body (*hd.*) projects conspicuously into the ganglion cells above and behind. It is not so large, however, as to make a prominence in the upper surface of that layer.

From the cells of the mushroom body (p. 119) the fibres pass into the head of the mushroom body. We must notice that there are no points at which a number of fibres enter together; the entry is general and spread over the whole surface of the head. Kenyon's application of the Golgi method to the brain of the bee makes it clear that after entering the substance of the head the fibres give off a collateral branch which in turn divides to form twigs. These twigs interlace with similar twigs from the collaterals of other cells to form a glomerular body (*Faserbällchen*). There must be several score of these bodies in the head of the mushroom body of *Micropteryx*. They are very small and by no means easily distinguished. After giving off its collateral the fibre proceeds downward as a component part of the stem. The fibres do not form a definite tract within the head, but pass through in a diffuse manner. It is only when they reach the underside that they unite to form the stem.

From the inner and inferior aspect of the head of the mushroom body a band of fibres sweeps downwards and

inwards past the side of the outer capsule of the central body. This band gives off a few fibres to the outer capsule (tract *n*), and then passes into that region of the protocerebral lobes which lies on each side of the central body (tract *r*); there the band divides and is lost to sight (Pl. VIII, fig. 12, and Pl. X). Such a tract has not been described in other insects.

The stem (*st.*) of the mushroom body leaves the ventral side of the head and passes downwards and forwards and also slightly inwards; it is a single cylindrical rod of parallel fibres; and whatever may be the case in other insects it is not penetrated by a canal. It may also be noticed that it is not surrounded by a sheath. (Even if it were I should not follow Kühnle in calling the sheath a neurilemma. A neurilemma is a syncytial layer covering a brain or ganglion.)

The stem is one of the most striking features of any section in which it occurs; it stains more heavily than the surrounding protocerebral lobes, and is a most useful landmark. The stem is a cylindrical structure, and well-fixed material shows that the greater part of its shaft is surrounded by a space which is not developed at its top or bottom; at these points the stem simply pierces the surrounding parts of the protocerebral lobes. At its lower and anterior end the stem is swollen and becomes superficial, that is to say it is no longer buried in the protocerebral lobes, but reaches the surface in the interval between the dorsal lobe and the anterior part of the ventrolateral lobe. At this point it is covered by a few nuclei; some of these are merely nuclei of neuroglia cells, some are nuclei of true nerve cells, which send their axons into the foot of the stem. This is certainly remarkable, but I have satisfied myself that it is the case by examining a large number of sections through this region.

At the bottom of the stem we should expect the roots to arise, and actually we find two processes of rather uncertain homologies, both of which make a marked angle with the stem. The first runs inwards and slightly backwards, and is the inner root (*in. r.; innere Wurzel* of Kühnle). It is straight and cylindrical and separated from its fellow of the opposite side by a very distinct part of the protocerebrum, the anterior part of the middle lobe. At its termination the inner root is somewhat swollen.

The second structure which leaves the anterior end of

the stem may be called the ascending trunk (*as. tr.*). The homologies of this organ are obscure and will be discussed later. It runs upwards for a very short distance and divides into two portions.

At its point of division it is swollen. For the moment let us call one of its branches the ascending and the other the posterior branch.*

The ascending branch (*as. br.*) runs up directly to the surface of the brain and is there swollen into an acorn-shaped head. It is covered by a thin layer of ganglion cells of the normal type, and these send their axons into the branch. This is a point of interest, for here and also at the foot of the stem we have a few nerve cells which appear to belong to the mushroom body. Similar conditions have been occasionally described in other insects, notably in *Periplaneta* by Haller.

The posterior branch (*po. br.*) of the ascending trunk runs backwards and slightly upwards and inwards. Its terminal part is bifid, but the two portions do not diverge from one another. It is completely enclosed in the protocerebral lobes, and even in fixed material is not always very easy to see. It is about two-thirds the length of the stem. Before we proceed to discuss the homologies of these organs it is absolutely necessary to grasp their anatomy and relationships.

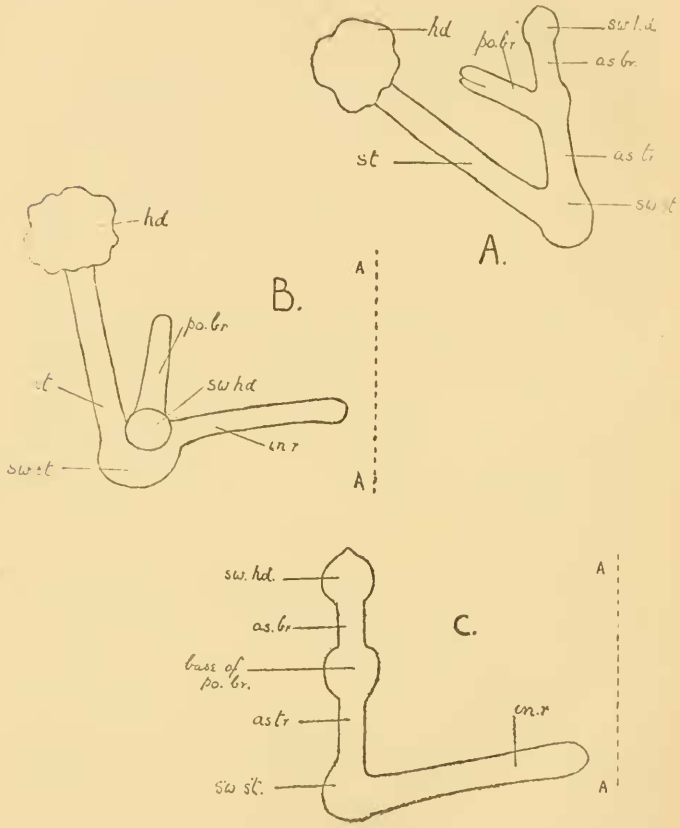
Let us now consider the homologies of these three branches of the stem. The first I have already identified as the inner root (*innere Wurzel* of Kühnle). This identification rests on its relations to other organs.

The ascending trunk is an organ for which I find no parallel in any insect brain yet investigated. This is not very remarkable when we remember that the brains of no *Lepidopteron* or *Trichopteron* have yet been fully described. According to a view which I now put forward the ascending trunk is to be regarded as the united base of the forward root (*vordere Wurzel*) and of the backward root (*rücklaufige Wurzel*). Great obscurity exists with regard to

* I use the term "trunk" and "branch" rather than "root" in order not to commit myself to any view as to homologies which are fully discussed later. The terms are of a provisional nature. It may be objected that I am adding to the synonymy, but it is almost a necessity to have some unequivocal name for an organ until its homologies are fixed. The word "root" I use as a full equivalent of the German *Wurzel*.

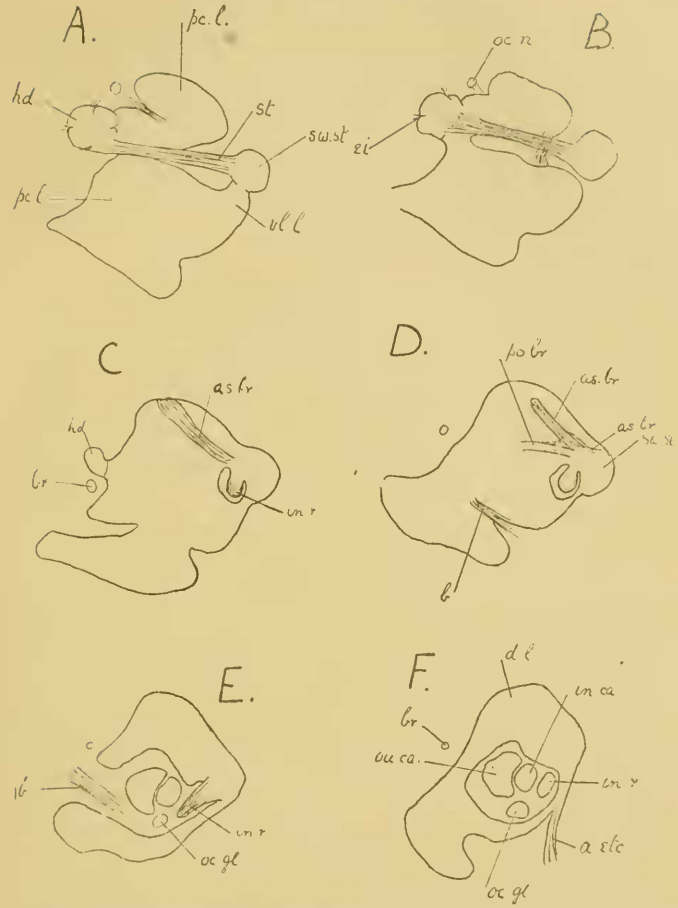
the homologies of the ascending and posterior branches of the ascending trunk.

It is probably best to consider the ascending branch as forward root (*vordere Wurzel*, Kühnle; *tubercule antérieur*,



Text figure 2.—Outline drawings of the right mushroom body. A, seen from the outer side (lateral view). B, from above. C, from in front. (The stem and head are behind and not shown in this drawing.) The line AA represents the median (sagittal) plane. The cells are not shown; the whole organ as drawn here consists of axonic substance.

as. br. ascending branch. *as. tr.* ascending trunk. *hd.* head of mushroom body. *in. r.* inner root. *po. br.* posterior branch. *st.* stem. *sw. hd.* swollen head of mushroom body. *sw. st.* swollen foot of stem.



TEXT FIGURE 3.—Outline drawing of six longitudinal vertical sections (A–F), to show the relations of the parts of the mushroom body to surrounding *pc. l.*, the last being nearest the middle line. The six sections are not consecutive. Dorsal is to the left; anterior (cephalad) towards the top of the page. Only the axonic parts are here shown, the cells being omitted.

a. etc. nerve fibres of tract *a* (p. 136) and other tracts, passing from protocerebrum to lower parts of brain. *as. br.* ascending branch. *as. tr.* ascending trunk. *b.* tract *b*. *br.* bridge. *d. l.* dorsal lobe. *ei.* Einströmmung (p. 120). *hd.* head of mushroom body. *in. ca.* inner capsule of central body. *in. r.* inner root of mushroom body. *oc. gl.* ocellary glomerulus. *oc. n.* ocellary nerve. *ou. ca.* outer capsule. *pc. l.* protocerebral lobes. *po. br.* posterior branch. *st.* stem. *sw. hd.* swollen head of mushroom body. *sw. st.* swollen foot of stem. *vl. l.* ventrolateral lobes.

Viallanes). This is suggested by its general direction and by the fact that it ends on the surface of the fibrillar part of the brain, under a thin portion of the ganglion cell layer. With this we may compare the similar "free" ending of the *tubercule antérieur* in Acridians (Viallanes, 1887, p. 42, fig. 46), and of the *vordere Wurzel* in *Apis* (Jonescu, p. 137, Text fig. 10*a*) and in *Vespa* (Viallanes, 1886). The author remarks: "*La première se porte directement en avant pour gagner la surface antérieure du renflement primaire; c'est la corne antérieure.*"

If, then, the ascending branch is the equivalent of the *vordere Wurzel*, we must homologise the posterior branch with the *rücklaufige Wurzel*, or backward root, in consideration of its backward direction and deeply buried termination: this would probably be accepted were it not that Kühnle has asserted that the *tubercule antérieur* of Viallanes is the homologue of the *vordere Wurzel* and also of the *rücklaufige Wurzel*. Against this I must enter a most emphatic protest. In the first place, there is an inherent improbability about it; we cannot willingly believe that *vordere* and *antérieur* refer to an organ which is described in other insects as "running back" (*rücklaufige*). We surely need good evidence before we can accept such a statement? In the face of that improbability Kühnle was dangerously bold in asserting the homology. When a great many more types have been investigated we shall be able to bridge many of the gaps which at present exist in our knowledge. Till then we can none of us be certain of any but the most obvious homologies, partly because the described types are so few, partly because no living man has first-hand knowledge of more than half a dozen insect brains.

Kühnle was probably led into this error by the fact that the majority of insect brains show only two roots, some of them lacking the forward, others the backward root.

If, however, Kühnle's homology be accepted, the one which I have suggested must fall; for clearly I cannot give the terms *vordere* and *rücklaufige* to two structures if, as Kühnle says, they are in this case synonymous. I do not wish to press my own convictions unduly; but at any rate they are based on considerations of relative position, that is to say on actual fact.

If, then, Kühnle is right and I am wrong, we may either assume that the ascending trunk and its branches (ascending and posterior) together form the backward (*rücklaufige*,

vordere) root, or else that the posterior branch represents that organ and that the ascending branch is a new organ. The first of these views is supported by the fact that the ascending and posterior branches leave the stem by a common origin, the ascending trunk. On the other hand, so far as our knowledge yet goes there is no other brain in which the backward root is bifurcated, which is what this view implies.

As I have said, it is also possible to regard the posterior branch as the *rücklaufige Wurzel* (*vordere Wurzel*, *tubercule antérieur*) and the ascending root as an organ which cannot be homologised with anything yet described. This is quite a rational view to adopt, for practically nothing is yet known about the brain of the Lepidoptera. Personally I do not see any necessity for dubbing this a new organ, but if Kühnle's identification of the *vordere* with the *rücklaufige Wurzel* is proved correct, then we shall probably find it necessary to find a name for what I have provisionally called the ascending branch. An investigation of other types might yield most fruitful results.

This very small and abstruse point must be settled definitely before the study of insect brains has gone further. Unfortunately it is not possible to attack the question from a comparative standpoint, but it is essential that we should start work with our homologies correctly and clearly defined. There can be no compromise between Kühnle's view and my own, and the point at issue is fundamental. I consider that there is, at any rate, very little reason for doubting my identification of the *innere Wurzel*, which agrees with the views of previous writers.

These conflicting views may be expressed thus : according to my view, ascending trunk = common origin of—

1. Ascending branch (*vordere Wurzel*, etc.)
   = Forward root.
2. Posterior branch (*rücklaufige Wurzel*)
   = Backward root.

Kühnle, however, asserts that *rücklaufige Wurzel* = *vordere Wurzel*. If this is so, then either—

(i) Ascending trunk and ascending branch and posterior branch together = *rücklaufige Wurzel*, or else—

(ii) Ascending trunk = common origin of (*a*) posterior branch (*rücklaufige Wurzel*) and (*b*) ascending branch (not homologous with anything yet described).

I believe that the characters by which the three roots may be separated are these: The inner root (*in. r.*; *innere Wurzel*, Kühnle; *tubercule interne*, Dujardin; *Balken*, Flögel) runs backwards and inwards and terminates between the middle lobe and the inner capsule of the central body. Its end is adjacent to that of its fellow on the opposite side. It appears that this root is found in nearly all insects. The forward root (*as. br.*; *vordere Wurzel*, Jonescu; *Vorderhorn*, Flögel; anterior root, Kenyon; *tubercule antérieur*, Dujardin) runs forwards and upwards and ends "free" on the surface of the protocerebral lobes, either under the ganglion cells or else directly beneath the neurilemma. The backward root (*po. br.*; *rücklaufige Wurzel*, Kühnle) runs backwards and terminates in the posterior part of the protocerebrum without ever reaching the surface. In many insects either the forward or the backward root is absent; this has led Kühnle to believe that they are identical.

I should like to take this early opportunity of answering one objection which will probably be made to the hypothesis that the mushroom body in its typical development possesses three roots. It is well known from Kenyon's work by the Golgi method on the brain of the bee, that the axons which compose the stem branch dichotomously, and that the two branches form the two roots of the mushroom body of that insect. Now it may be urged that this division of the axon into two, which is probably characteristic of the nerve cells of the Arthropoda in general, would find its outward expression in a mushroom body with two roots. To this I may, however, reply that there is no difficulty in supposing that each fibre as it divides supplies two of the three roots; and at any rate the difficulty remains whether the ascending and posterior branches be one root or two, for the plain facts of their anatomy can hardly be disputed. Furthermore, we are already familiar with the division of the roots in the brains of other insects; now if the fibres which compose a root can be grouped in such a way as to produce a bifurcation of the root, why should not the fibres of a stem be so grouped as to supply almost any number of roots? Moreover, Kühnle has already described the mushroom body of a Phasmid, and of a Termite, both of which had three roots, though he failed to grasp the bearing of this fact upon the general question of homology. The fact of the existence of three roots to the mushroom body is

not, then, a new discovery, but I trust that I have been enabled to put the homologies of the matter on a sound basis.

IV. The Central Body.

The central body of *Micropteryx* consists of two capsules; the larger of these, the outer (*ou. ca.*), is superior and posterior, the smaller or inner capsule (*in. ca.*) is inferior and anterior. They are respectively the *äussere Schale* and *innere Schale* of Kühnle. They lie together in that space in the middle of the protocerebral lobes to which Viallanes gave the name *la loge*; this contains also the ocellary glomeruli and the inner roots of the mushroom body (Pl. VIII, figs. 7–10). The space is bounded on all sides by the protocerebral lobes, and above by the tract *f*, in front by the tract *c*, behind by the tract *d*, and below by the double tract *e* (see p. 137).

Micropteryx is one of those insects in which the central body is large and the mushroom body comparatively small; that is to say, it falls within one of Bretschneider's lower categories.

The outer capsule is slightly wider than the inner. The anterior edge of each is in the same vertical plane, but the outer extends back a considerable distance behind the inner, and this posterior part of it is very thick; thus the outer capsule overlaps the inner above and behind and is much the more bulky of the two. This condition is characteristic of nearly all the insect brains which have yet been described. Turning to internal structure we find that the outer capsule stains rather more deeply with eosin or orange G than do the protocerebral lobes. There is no definite division of either capsule into bodies like the rays of a fan, a condition which has been described in the brains of various insects since the time of Dietl. The anterior part of the inner capsule is, however, divided into a number of small rounded masses arranged in no definite manner and separated from one another by bands of axons, the great majority of which pass into the outer capsule. These masses resemble to some extent the glomerular bodies (*Faserbällchen*) of the antennary lobe. The scattered neuroglia cells which lie in the space which surrounds the central body are referred to elsewhere (p. 120). There is no group of cells which can be said to belong to the central body either here or in any other insect, and we believe that

the organ is a reflex centre not connected with any one motor or sensory function.

(The ocellary glomeruli, which are sometimes considered with the central body, are described on p. 135.)

V. The Bridge.

(Die Hirnbrücke—Kühnle, etc. Le Pont—Viallanes. Fibrillar Arch—Kenyon.)

The Bridge (*br.*) is a protocerebral structure found in all insect brains. In *Micropteryx* it occupies its usual position as a transverse band of axonic material on the superior side of the protocerebrum. It lies behind the nerve to the ocellus, and between the heads of the mushroom bodies (Pl. VIII, IX and X).

The bridge consists in part of Punktsubstanz; this is found at either end. The middle of the bridge is much attenuated and appears to consist entirely of nerve fibres passing from one side to the other. The organ, then, is dumb-bell shaped, the swollen ends, or "heads," being presumably centres, the narrow waist a decussating tract. The bridge is covered by some layers of ganglion cells, which appear to belong to the normal type.*

The axons of these apparently "normal" cells pass downwards to several parts of the brain. Some which proceed from the more lateral cells pass in front of, or behind, or round the end of the bridge, and enter the dorsal surface of the protocerebral lobes in a diffuse manner. Other axons pass through the heads of the bridge and so onwards to the lobes of the protocerebrum; it is probable that these give off a collateral while they are within the substance of the bridge. Other cells, again, send their axons into the bridge itself, where the fibre is lost to sight. It is probable that some of these fibres cross the middle line. The whole matter requires investigation by the Golgi method. We have, then, a number of similar cells, some of which appear to be associated with the bridge, some with the dorsal part of the protocerebrum, some, again, with both. From this I am inclined to argue that the bridge is of less importance as a physiological entity than some authorities have believed; that it is rather of anatomical than of

* In *Forficula* Kühnle distinguishes several types of cell in this region, but in every respect the ganglion cells of *Micropteryx* appear to have very little tendency to be differentiated into types.

physiological significance. Against this view is the admitted fact that the bridge exists as a distinct structure in all insect brains which have been investigated.

I can find no visible connection between the bridge and the nerves supplying the compound eyes, though a few fibres of the ocellary nerve enter the ends of the bridge. This perhaps supports Kühnle and tends to contradict the contention of Bretschneider and others who regard the bridge as a centre for the co-ordination of visual impulses.

VI. The Visual Centres.

A. *The Ocellary Apparatus.*

In *Micropteryx* paired ocelli are present, but the median ocellus is not developed here, or in any other Lepidopteron or Trichopteron. A stream of fibres, the ocellary nerve (*oc. n.*), leaves the back of the spherical chitinous capsule in which the ocellus is contained. At the point where the fibres leave the capsule there is some tendency for the nerve to break, as it is very much narrowed. The sensory cells are contained partly in the capsule of the organ, and some of them lie along the course of the nerve away from the actual ocellus, and as the nerve proceeds inwards they become less and less numerous. The nerve runs straight towards the middle line in a plane slightly anterior to the head of the mushroom body (Pl. VIII, figs. 10 and 11). When it is over the external edge of the central body it bends backwards, and at this point a few fibres leave it to pass into the protocerebral lobes. From here it passes backwards and inwards and continually gives off more and more of its fibres, so that though there is no point at which the ocellary nerve as a whole passes into the substance of the protocerebral lobes yet the whole nerve ultimately does so. A few fibres may also be seen to pass into the swollen head of the bridge.

Two small spherical bodies with rather indefinite margins are found in the space beneath the outer capsule of the mushroom body, posterior to the inner capsule and to the middle lobe (Pl. VIII, fig. 10); these are the ocellary glomeruli (*oc. gl.*) or "*tubercules du corps central*" (Viallanes). In *Micropteryx* I have been unable to demonstrate the connection between these structures and the ocellary nerve, owing to the diffuse way in which the fibres of the nerve pass through the dorsal part of the protocerebrum.

From a consideration of what is known about similar organs in other insects I do not consider that much doubt exists as to the functional connection between these small, round, deeply placed lobes and the ocellus. It is hardly to be expected that we should be able to find more than a proportion of the smaller tracts in so minute a structure as the brain with which we are dealing. Fibres pass from the ocellary glomerulus to many parts of the brain, including the paired (deuterocerebral) sympathetic system (tract *s*) and the posterior part of the antennary lobe (tract *t*).

B. *The Optic Lobes.*

Pressure of other work has absolutely prevented my devoting attention to the optic lobes (*o. l.*), and much special study of the literature would be required before I could hope to treat of them at all adequately; this would delay the publication of this paper almost indefinitely, and I have accordingly decided to leave them entirely undescribed rather than to deal with them in an inadequate manner.

VII. The Protocerebral Tracts.

Authors have frequently attempted to enumerate the tracts of fibres which connect one part of the brain with other parts, but they can only detect and describe the larger bundles and can never even attempt to follow the minute tracts which ramify in all directions through the Punkt-substanz. Such an enumeration must always be incomplete even if one part of the brain is proved to have a score of paths connecting it with other regions : and even if the Golgi method is applied to a very large number of individuals, definitive completeness can hardly be arrived at. In this present paper I make no attempt to give any complete list : I only describe a few of the more noticeable tracts which are useful either as landmarks or else as definite boundaries to regions.

The following tracts should perhaps be noticed, because they are important features of the sections in which they occur. (*a*) A wide tract of fibres arising from cells situated over the dorsal part of the protocerebrum, and passing vertically downwards in the middle line over the front of the protocerebral lobes : this tract forks below and the fibres then pass to the antennary lobe of each side, and

possibly also to the tritocerebrum and the ventral parts of the central nervous system (Pl. VII, figs. 2–4).

(*b*) A tract of fibres which is the *Riechstrang*, or *Riechbahn*, of the German authors. The cells from which the tract arises appear to be ganglion cells of the "normal" type, and are situated dorsal to the protocerebral lobes and slightly behind the head of the mushroom body: the fibres pass downwards and forwards and slightly inwards, through that part of the protocerebral lobes which surrounds the space containing the central body, etc.; from here the fibres are directed downwards and outwards to the antennary lobe. Through most of their course the fibres lie in a free space. Owing to its diffuse structure, which renders it difficult to distinguish it when it is cut transversely, the lower part of this tract can only be distinguished in longitudinal section (text fig. 3, D and E, p. 129).

(*c*) A broad anterior commissure (Pl. VII, fig. 6).

(*d*) A deep commissure.

(*e*) A double ventral commissure (the *vordere und hintere Brücken der Nebenlappen unter sich* of Kühnle; Pl. VIII, fig. 9). These three are the most definite tracts which unite the two sides of the protocerebrum, though it should be remembered that the lobes are united over the greater part of their internal aspects, and that a large number of smaller tracts pass from one side to the other. These three tracts form the boundary of the "*loge*" in front, behind and below: the anterior and ventral ones lie upon the surface of the fibrillar part of the brain, the deep commissure passes between the two protocerebral lobes behind the "*loge*": the ventral commissure (*e*) arises on each side from the anterior part of the ventrolateral lobe.

(*f*) A small but distinct band of fibres which runs transversely across the upper surface of the "*loge*." This tract is the *Faserhof* of Kühnle, and possibly also the *commessura protocerebrale anteriore* of Berlese (Pl. VIII, fig. 8).

(*g*) A posterior commissure uniting the two protocerebral lobes at their most posterior part, where they overlap the tritocerebrum (Pl. IX, fig. 16).

(*h* and *i*) Tracts uniting respectively the anterior part of the middle lobe to the anterior part of the ventrolateral lobe, and the posterior part of the middle lobe to the posterior part of the ventrolateral lobe of the same side.

(*j*) A tract running from the posterior part of the middle lobe to the deuterocerebrum.

(*k*) A tract running up from the middle lobe into the deeper parts of the protocerebral lobes which lie lateral to the "*loge*."

(*l–q*) Tracts from or to the central body.

(*l*) The two capsules of the central body are united especially along their anterior margin by fibres which make the partitions between the "*Fächer*" of the inner capsule (Pl. VIII, fig. 8).

(*m* and *mm*) Both capsules are united to the protocerebral lobes by bands of fibres which leave their anterolateral margins; the band from the upper capsule (*m*) passes upwards, that from the lower (*mm*) downwards to the ventrolateral lobes (Pl. VIII, fig. 7).

(*n*) The outer capsule receives fibres which leave or possibly enter the head of the mushroom body on its under side. This is really a part of tract *r*.

(*o*) A few fibres connect the outer capsule to the bridge.

(*p*) A few also pass from the ocellary nerve to the outer capsule.

(*q*) A well-marked band connects the antennary lobe with the outer capsule. This band enters that part of the outer capsule which lies immediately superior to the posterior part of the inner capsule (Pl. VIII, fig. 10).

(*r*) The tract which has been mentioned on p. 126 as leaving the inner and inferior aspect of the head of the mushroom body, and passing partly to the outer capsule of the central body (tract *n*), but mainly to the deep part of the protocerebral lobes lateral and posterior to the "*loge*" (Pl. VIII, fig. 12; Pl. IX, fig. 13; Pl. X).

(*s* and *t*) These tracts pass from the ocellary glomerulus to the paired deuterocerebral sympathetic system and to the posterior portion of the antennary lobe respectively.

(*u*) This consists of a few fibres which pass down from the swollen ends of the bridge to the dorsal lobe immediately below and to the tumulus (Plate X).

It is, I believe, generally true that paired organs are united across the middle line, but I am unable to say whether this is invariably the case.

Summary.

The protocerebrum of *Micropteryx* might be described in the following terms. The neurilemma, which covers the whole central nervous system in one continuous sheet,

is a thin syncytium, and beneath it are found the ganglion cells and the axonic parts of the nervous system. Over the protocerebrum the layer of ganglion cells is deep, and four types can be distinguished: the normal type, the mushroom body cells, the cells of the optic lobes, and the giant cells. Neuroglia cells are found in the substance of the protocerebrum in small numbers. The tracheal system of the brain is very slightly developed. The protocerebral lobes are large, and in volume greatly exceed the other parts of the protocerebrum together. The various parts of the protocerebral lobes which have been described in other insects are all present, though *Micropteryx* presents some peculiarities, for the ventrolateral lobe and the middle lobe are each divided into anterior and posterior portions. A mid-dorsal lobe is also present, and to this I have given the name tumulus, an organ which has not been described before. The mushroom bodies are of a small, simple type, and only one is developed on each side: *the head is remarkable because of the shape, which is that of a rough sphere, without any approach to the formation of a cup.* In section it is seen to contain minute glomerular masses of nerve fibres, which are regarded as association centres: these are comparable to similar structures described in the mushroom bodies of many insects, and also in antennary lobes and central bodies. The origin of the stem is below, not within, the head of the mushroom body, and it runs downwards and forwards in a definite space; it is rod-like, and not perforated by a canal. The stem divides below in a complicated manner which does not lend itself to summarisation. I have suggested several possible homologies for the parts into which the stem divides, and my own view is that there are *three roots* to the mushroom body in this insect—an *inner*, a *forward*, and a *backward*—and that this is the typical number for the insect brain: other views are also discussed. I have also given what I believe to be the normal relations and characters of these roots; and I believe that this part, at any rate, of my paper has some permanent value. *The central body is large*, and consists of two capsules, as usual; the outer is the larger. *There is no tendency towards the division of either capsule in a fanlike manner*, but the inner capsule contains a number of minute glomerular bodies. The tracts passing from or to the central body are numerous and some of them are large. The nerves from the ocelli run inwards

across the front of the head of the mushroom body and pass gradually into the substance of the protocerebral lobes, and a few fibres pass into the bridge. Two small bodies are found beneath the central body, and these are presumed to be the ocellary glomeruli of other authors, though in the brain of *Micropteryx* there is no actual evidence of their connection with the ocellary nerve. The bridge is simple and straight; its ends are rounded and consist of Punktsubstanz, and into these pass the axons of a few cells which are situated in the immediate neighbourhood; the middle of the bridge is formed of a large number of fibres which pass across the middle line. (I have underlined those characters which appear to indicate that the brain of *Micropteryx* belongs to a simple type, so far as morphological points are concerned.)

It would doubtless be interesting to compare the simple brain of this Protolepidopteron with that of other *Lepidoptera* or *Trichoptera*. This is, however, impossible, except to a very slight degree, because the only work to which we can refer is the classic paper which Flögel published in 1878, and a few lines in Berlese's text-book. Flögel dealt with the brains of a number of larvae and imagines of *Lepidoptera*, and his fullest description is that of the brain of the imago of *Cossus*. He devotes his attention to the mushroom body, which differs from that of *Micropteryx* in several important particulars. The head is developed as two cups on each side, placed in apposition to one another. The two stems which proceed downwards from these unite to form a single cylindrical stem which stains deeply and lies in a space. An inner root is given off, and this occupies the usual position of that organ; there is also a forward root which runs up to the surface of the brain and there divides in a complicated manner which is not further described; no backward root is described, but it is possible that this is represented by one of the branches of the forward root. This suggestion is an attempt to bring *Cossus* into line with *Micropteryx*, and it may well be correct, for we must remember that Flögel was hampered by the deficient methods of his time, and that he was the earliest insect neurologist in any true sense of the word.

Berlese describes the brain of *Sphinx* very shortly. The protocerebral lobes are large, the mushroom bodies of moderate size; two pairs are present, which lie one in front of the other: their stems do not unite. A mass of

very large cells (*cellule maestre*) are developed behind and above the protocerebrum, and the fibres from these proceed over the front of the protocerebrum to the ventral brain by way of the œsophageal connections.

TECHNIQUE.

I. Fixation and Impregnation.

My early work on *Micropteryx* was all done upon material which had been fixed and stained by very simple methods. I became convinced that for insect neurology the employment of complicated technique was not only desirable but necessary. Accordingly I devoted the early spring of 1915 to a somewhat extensive series of experiments in staining and fixing the brains of cockroaches (*Periplaneta*), my object being to familiarise myself with some forms of technique which I proposed to apply later to *Micropteryx*. I shall describe my methods for both insects together, though some of them are only applicable to one or other of the insects.

Owing to the chitinous cuticle of insects it is necessary to take every care to ensure the penetration of the fixing fluid. Unless there is good reason to the contrary *Micropteryx* should be cut in two with a sharp knife; only the anterior end will be preserved and fixed. A cockroach, on the other hand, should be chloroformed and held between the finger and thumb, with the head resting on the thumb-nail; the epicranium should then be punctured with a small sharp knife, and also the eyes if the individual is a large one; the same knife should then be used to remove all the mouth parts and the labrum at one transverse sweep, the thumb-nail forming a block on which the cutting is done. All this can be performed without any damage being done to the brain by pressure. The head is then cut off and placed in the fixing fluid.

Fixatives. Osmic Acid (osmium tetroxide). — This is perhaps the most generally used of all fixatives, ever since the time of Viallanes, who described it as "*le réactif le plus précieux que nous possédions pour mettre en évidence le trajet des fibres.*" It has been used in strengths of from ¼% to 1%. Flemming's solution has also been much used, and it is probable that its results are slightly better than those given by osmic acid alone. Böttger recommended its employment for periods of about three weeks;

I cannot see that anything is gained by leaving material in it for so long a time, though it is well known that all fixatives containing osmic acid penetrate slowly even through small pieces of tissue. Forty-eight hours is quite sufficient, according to my experience. Borrel's fluid also gives good results very similar to those obtained with other osmic acid fluids. *Micropteryx* tends to float in this and other fixatives; if it cannot be caused to sink with the aid of shaking it may be lightly painted with 90 % alcohol in order to reduce the surface tension. All these fixatives are extremely useful, though they occasionally tend to shrink the cytoplasm of the larger nerve cells. The nerve fibres (axons) stand out from one another with great clearness, and in this way the sections are well suited for study: they are never distorted, and there is no tendency for the ganglion cells to break away in masses from the underlying fibrillar substance. Great care must be exercised in washing the material very thoroughly in water after fixation, or the staining will be unsatisfactory.

Formalin.—Formalin has been recommended in various rather high percentages (10 %, 20 %, etc.) by more than one worker. It is customary to leave the heads in it for some days. I anticipate that the use of formalin will soon be discontinued, for though it gives a distinctly good demonstration of the tracts of axons, there is a great tendency for the formation of vacuoles in the fibrillar substance. The result of this is that the tracts are pushed to one side and distorted. This vacuolisation is not invariable, but it constitutes a grave defect in the method, which is one that I found unreliable.

Picro-Chlor-Acetic Mixture. — I do not know to whom we are indebted for this very useful fixative; but it appears that it has not previously been used by insect neurologists. My own experience is that it is the best general fixative I have ever employed, and I trust that the workers of the future will be as satisfied with it as I am myself. It possesses very great powers of penetration, and can be relied on to fix small insects completely without decapitation or any other precautions. Insect histologists will find that it is an exceedingly fine preservative of the details of cell-structure; as far as the brain is concerned this fluid demonstrated the tracts of axons with particular clearness, and in this respect it does not fall far short of osmic acid. The nerve cells are also well preserved, and

all the different types can easily be distinguished, though for a special study of the cells it is certainly best to have some material fixed for that purpose in Bouin's fluid.

Bouin's Fluid.—This fixative is only of use for a study of the nerve cells, and for this purpose it is unrivalled. It fixes material in such a way that the tracts of fibres cannot be distinguished at all, but that is immaterial provided it is realised that the fluid is essentially a special fixative.

Acetic Sublimate Solution.—This is simply a saturated solution of mercuric chloride in dilute alcohol to which a small percentage of acetic acid has been added. It has been used by other authors but there is nothing to recommend it; the tracts or bundles of axons are shown in much the same way as they are in material fixed in the picro-chlor-acetic mixture, the cells are shrunken and the different types cannot be distinguished, and the fluid has poor power of penetration.

Perenyi's Fluid.—This is a fixative with very small power of penetration, even when used hot. Even if penetration is secured the tracts of axons cannot be distinguished from one another, and the cells are swollen and matted together.

Gilson's Fluid.—The penetrating power of this fluid is so great that insects may be fixed in it whole. It is an excellent fixative of ganglion cells, and shows the differences between the types very clearly: for this purpose it is valuable but it fails entirely to define the axons.

Bichromate. — Potassium bichromate, apart from its use in the Golgi method, is quite useless as a fixative of insect nerve tissue, first because details of structure and the course of axons are not well preserved, secondly because material so fixed stains most intensely and generally with the aniline dyes, thirdly because these stains can scarcely be washed out or differentiated, and fourthly because of the great brittleness of sections which have been exposed to the action of these fluids.

The Golgi Method. — This method has been applied by Kenyon to the brain of the bee, and with it he has obtained some very remarkable results; his original paper (Kenyon, 1896, I) should be consulted for a full account of his procedure. It is almost impossible to apply it to insects which are not available for the greater part of the year because it is extremely precarious, and even Kenyon himself only obtained good results with an occasional

specimen. I have been unable to use it with any success upon *Micropteryx*.

Metallic Impregnation.—The object of this method is to impregnate nerve cells and fibres with actual metallic silver and gold. The silver salt which is invariably used is the nitrate, and as it gives excellent results I have tried no other salts. The heads are dropped into a solution of this salt and kept in the dark for a period. I have devoted some time to discovering the best strength of silver solution and the period during which the heads should be exposed to its action. I find that the best results are obtained by dropping them into 1 % silver nitrate in water, and leaving them in the dark for ten days. The silver is very slow in penetrating the head, and if a 6 % solution is used there is great danger that the periphery will be blackened before the central portions are affected at all. I believe that penetration can be accelerated by keeping the whole at 30°–35° C. It is probable that the period during which the head lies in $AgNO_3$ is immaterial provided that the fixation proceeds in the dark and that sufficient time is allowed for the full and equal penetration of the silver. It was not found advisable to assist the silver to penetrate more quickly by employing an alcoholic solution of the salt. I have, for instance, experimented with a 1 % solution in 30 % alcohol, following this by the various processes which I describe below. The impregnation of the various fibres was not obtained at all, though the various parts of the brain were coloured to different degrees. In fact, the alcoholic solution of silver gave quite a pretty differential stain of no particular value, but failed utterly to produce the sweeping black lines which are what is desired.

The head, then, is fixed for ten days in 1 % silver nitrate in darkness. It is then washed. A few workers transfer it to pyrogallic acid for a day, in order to reduce the silver and leave it in the tissues in a finely divided state. I am quite convinced that this is unwise. The reduction may be done much more evenly by a method which I shall now describe. The heads are embedded, unreduced, in paraffin, fixed to the slide in the usual manner, and treated with xylol and descending grades of alcohol. At this stage the sections are sienna-brown in colour. From a low grade of alcohol the sections are moved to distilled water. (I need hardly say that if the heads or sections are brought into tap water a fine deposit of chloride will be precipitated which

will completely ruin the preparations.) The slides are now placed in 1–2 % $AgNO_3$ and exposed to bright sunlight or an electric lamp for about ten minutes. After this they are washed for two minutes in distilled water and placed in 1 % gold chloride for two minutes in a bright light. They are then again washed and placed in an aqueous solution of pyrogallic acid until the reduction is complete, deposits of metal being left in the fibres. The sections are now brought up through the usual grades of alcohol, stained for a very few seconds in orange G, and mounted. These preparations do not degenerate under the cover-slip in the same manner as Golgi preparations.

This impregnation is only a modification of one introduced by Ramon y Cajal; a similar method has been employed by Jonescu.

If it is successful it gives sweeping black lines of axons running through the brain in the most diagrammatic manner. It is unfortunately almost inapplicable to so small an insect as *Micropteryx*, owing to the fact that the aqueous silver solution hardly penetrates the insect's minute neck even after decapitation. I am quite confident that this method will be found most useful in the study of the brains of insects which are large enough to admit of the brain being laid partly bare to the fixing fluid.

II. Section Cutting.

All material should be stored in 90% alcohol, rather than in a lower percentage. Excellent material may be completely ruined if the spirit in which it is kept has ever been in contact with cork, the tannin of which interferes with the action of most stains: glass-stoppered vessels must accordingly be used.

Section Cutting.—It is well known that the cutting of sections through heavily chitinous insects presents great difficulties. Much may be done to overcome this, but before discussing methods of softening chitin I should like to state my firm conviction that the one factor of prime importance is the microtome knife. In the absence of a really sharp knife no softening reagents and no care exercised during the embedding are of the slightest value. The best softening reagent, so far as my limited knowledge goes, is spirit soap (German Pharmacopœia), the use of which was first advocated by Kurt Bedau. The insects are placed in

this for some days, well washed in 70 % and 90 % alcohol, and then embedded. The chitin is certainly softened by this solution, but will regain some of its hardness if it is simply embedded in hot paraffin. It appears that heat, absolute alcohol, and xylol, all exercise a marked hardening effect on chitin. It is best, then, that the head or insect should be dehydrated as much as possible in 90 % alcohol, left a short time in absolute alcohol, and cleared in chloroform. I then place it for some days in a chloroform solution of paraffin, and finally drop it into the hot paraffin of the ordinary embedding bath. Here it remains only long enough for it to attain the temperature of the bath, and is then removed in the crucible or other vessel in which the paraffin is contained, and placed under a vacuum pump; the pump will quickly remove the chloroform, most of which has by now become diffused into the paraffin. The mass may then be turned out into a mould and cooled. Terpineol has also been used as a softening reagent and it appears quite satisfactory, though I have not much experience of it.

Celloidin.—I have used this to some extent, though I no longer do so, because I find it unnecessary if spirit soap is used as described above. It cannot be relied upon to penetrate a whole insect unless thin celloidin be employed for many days.

Both with and without celloidin I have been able to obtain serial sections of the head of *Micropteryx* of considerable thinness. I have several series of 3·5 μ, which is not by any means too thin, because of the smallness and complex structure of the brain.

Practical experience teaches me that it is never safe to move either complete brains or sections from absolute alcohol to xylol or vice versa, but that an intermediate mixture of the fluids should always be employed. Unless this is done the ganglion cells will frequently break away from the axonic part of the brain.

III. Staining.

For general study sections should be stained with Delafield's hæmatoxylin, and orange G (eosin may also be used, but I think that the orange G gives better results). Such sections are excellent for preliminary work, and I always use this stain as a standard test for a fixative which is new to me.

Another valuable stain is PICRO-NIGROSIN. This brings into special prominence the tracts of fibres which run through the brain. Counter-staining should be avoided, and also over-staining, because picro-nigrosin washes out only with difficulty in acid alcohol. The best results are obtained with material fixed in osmic acid or Flemming's solution.

Other hæmatoxylin methods have little to recommend them. The Weigert-Pal method cannot be used because it is specific for myelin sheaths, which are never found on the nerve fibres of insects.

5% HÆMATOXYLIN CONTAINING LITHIUM CARBONATE is a stain for nerve fibres, but picro-nigrosin gives similar though more distinct results. Staining with MALLORY'S HÆMATOXYLIN is very strongly recommended by Kenyon and by Altens. The process is complicated. At first sight the stain appears much too general to be of use. Every tissue is stained a heavy dark blue, and differentiation in acid alcohol is useless. I have discovered that the sections may be quickly differentiated in a solution of sodium bicarbonate in distilled water. The stain becomes bright sky blue and most of the tissues are partly decolorised. The nerve fibres retain the stain. This complicated method produces results which are not really superior to those produced by staining with picro-nigrosin. The blue colour appears to be permanent. The cytoplasm of the giant cells retains even more of the stain than do the nerve fibres. The ordinary small ganglion cells are very much decolorised in the alkali.

MALLORY'S ANILIN BLUE. — This has been used by Bretschneider in his work on the brain of *Periplaneta*. The method is one of great complexity, especially with the addition of the modifications which he introduces. The results are extremely beautiful, but most workers will probably consider them hardly worth the trouble and time expended on them.

MANN'S STAIN.—I am much indebted to Dr. D. Keilin for insisting on my giving a trial to this stain, which will be found of great value. I find it best to stain first, lightly, with Delafield, but this is not necessary or desirable except for nerve cells; the stain is widely known to insect histologists, and is strongly recommended to neurologists. It is seen at its best when applied to material fixed in the fluids of Bouin, or Gilson, or in the

picro-chlor-acetic mixture; it should not be used upon osmic acid preparations.

I have tried several stains which have proved more or less useless, and I mention them below in order to save others from wasting time upon them.

Methylene Blue and Methyl Violet.—It appears that the cells have little affinity for these stains. This is remarkable when it is remembered to how large an extent methylene blue has been used as a vital stain for the nervous systems of the Arthropoda.

Van Gieson's Stain.—This stain is useless because it colours all the soft parts of the section a uniform pink colour, without any of the differentiation which it gives with sections of the tissues of Vertebrates.

Various preparations of carmine were tried, because of its historic interest as the only stain used by the workers of thirty or forty years ago. It appears to have singularly little affinity for any part of the brain of *Micropteryx*.

The stains on which I place most reliance are Delafield's hæmatoxylin with orange G as counter-stain for preliminary study, picro-nigrosin and the reduced silver and gold method for the study of the course of nerve fibres, and Mann's stain for the nerve cells.

IV. Note.—Some of the fixatives and stains to which I have had reason to refer are not very well known, and it will perhaps be helpful if I give their compositions. The *picro-chlor-acetic* mixture is 1 % picric acid in absolute alcohol, 6 parts; chloroform 1 part; formalin (40 %) 1 part; glacial acetic acid $\frac{1}{2}$ part. Fix twenty-four hours, then three days in 90 % alcohol. *Borrel's fluid* consists of osmic acid ($Os\,O_4$) 1 gm.; acetic acid 10 c.c.; platinum chloride 1 gm.; chromic acid 1·5 gm.; and distilled water 175 c.c.

The *spirit soap* which is recommended as a reagent for softening chitin is one of the official preparations of the German Pharmacopœia: 6 gms. of olive oil are saponified with 7 gms. of a solution of potassium hydroxide; to this is added alcohol 30 gms., water 17 c.c. (Kurt Bedau. Zeitschr. f. wiss. Zoologie, Vol. 97, p. 418, 1910–11).

Of the stains the following should perhaps be described. The *picro-nigrosin* I used was made up as follows: 1 vol. 1 % aqueous solution of nigrosin; 9 vols. saturated aqueous solution of picric acid. The fact that the various authors

who have tried this stain give conflicting accounts of its value is due to the fact that there is no standard composition for it. *Mallory's hæmatoxylin.*—The stain consists of hæmatoxylin crystals 1 gm.; chloral hydrate 10 gms.; 10 % solution of phosphomolybdic acid in water 1 c.c.; distilled water 100 c.c. The sections are mordanted in 5 % copper sulphate solution for twenty-four hours, washed in tap water, placed for ¼ or ½ hour in the stain diluted with four times its volume of distilled water, rinsed and carefully decolorised in a solution of sodium bicarbonate in distilled water. Bretschneider's application of *Mallory's anilin blue*, with some slight modifications of my own, is as follows: Delafield's hæmatoxyl 1 hour, or until the nuclei are faintly stained; wash; eosin twenty minutes; wash in water; 1 % phosphomolybdic acid two minutes. Mallory's stain one minute; wash, dehydrate and mount. The composition of the Mallory's stain is anilin blue (water-soluble) ½ gm., orange G (water-soluble) 2 gms., oxalic acid 2 gms., distilled water 100 c.c. *Mann's stain;* 1 % aqueous sol. methyl blue 35 c.c., 1 % aqueous eosin 35 c.c., water 100 c.c. Stain ten minutes or longer, for it is impossible to overstain, and then wash in alcohol containing 1 % of pyridin, watching the process of decolorisation under the microscope; with practice the right degree of decolorisation can easily be obtained.

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A very complete bibliography is given by Kühnle. It is only necessary for me to give a list of the authors and papers to which I have referred.

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Abbreviations Used.

In every case the same abbreviations are used in the Plates as in the text figures.

The letters *a*, *b*, *c*, *d*, etc., refer in all cases to tracts.

a, *b*, *c*, *d*, etc. (p. 136).
as. br. = ascending branch (= forward root) of mushroom body.
a.s. = axonic substance = fibrillar substance.
as. tr. = ascending trunk of mushroom body.
br. = bridge.
br. hd. = head of bridge.
d. l. = dorsal lobe of protocerebrum.
dm. = deuterocerebrum (= antennary lobe).
d. sy. = deuterocerebral or paired sympathetic system.
ei. = Einströmmung (see p. 120).
g. c. = ganglion cells of the " normal " type.
gi. c. = giant cells.
hd. = head of mushroom body.
in. ca. = inner capsule of central body.
in. r. = inner root of mushroom body.
l. l. = lateral protocerebral lobes.
lo. = " la loge " of Viallanes.
mb. = mushroom body.
mb. c. = cells of mushroom body.
mi. l. = middle lobe.
mi. l. a. and *mi. l. p.* = anterior and posterior portions of middle lobe.
mm. = tract *mm.* (p. 138).
mo. n. = motor antennary nerve.
ng. = nuclei of neuroglia cells.
nl. = neurilemma.
nn. = nuclei of the neurilemma.

o. c. = cells of optic lobes.
oc. gl. = ocellary glomerulus.
oc. n. = ocellary nerve.
o. l. = optic lobes.
ou. ca. = outer capsule of central body.
pc. l. = protocerebral lobes.
po. br. = posterior branch (= backward root) of mushroom body.
s. n. = sensory antennary nerve.
st. = stem of mushroom body.
sw. hd. = swollen head of ascending branch.
sw. st. = swollen foot of stem.
tm. = tritocerebrum.
tr. = tracheal tubes.
tu. = tumulus.
vl. l. = ventrolateral lobe of protocerebrum.
vl. l. a. and *vl. l. p.* = its anterior and posterior portions.

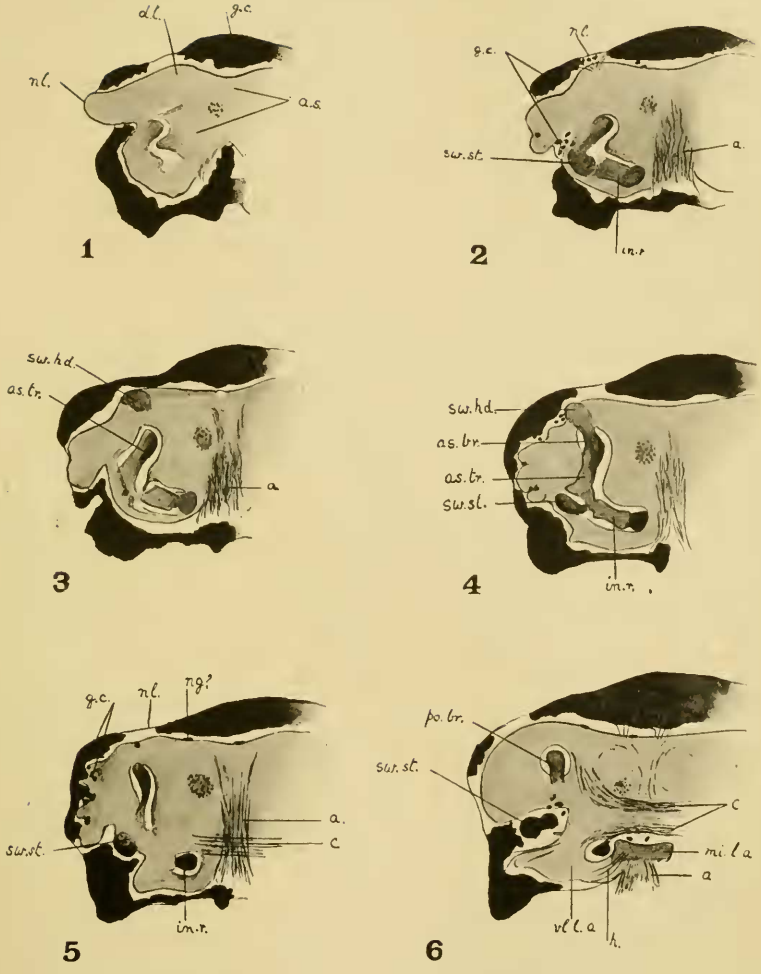
Explanation of Plates.

Plates VII to IX (figures 1 to 16) represent a series of vertical transverse sections through the protocerebrum of *Micropteryx*, partially diagrammatic. Each figure represents a successive section 3·5 μ thick, except that single sections are omitted between figures 6 and 7, 9 and 10, 14 and 15, and 15 and 16.

The neurilemma is shown as a black line, the cells of the mushroom body (*mb. c.*) and those of the optic lobes (*o. c.*) by black masses, and the normal ganglion cells (*g. c.*) by dark grey, except where one or two occur alone, in which case they are represented as individual black dots (*c. g.* fig. 5). Giant cells (*gi. c.*) are always drawn individually. Axonic substance is shown pale grey, except the stem of the mushroom body, which stains heavily and is coloured dark, and certain other parts of the protocerebrum which are shown in medium grey because they stain slightly more intensely than other parts. Bands of fibres are shown as dark lines when they are striking features of any section.

The upper part of each figure is dorsal, the lower ventral.

Plate X (fig. 17).—This plate is from a thin section, hence the amount of free space among the ganglion cells. The drawing re-



BUXTON: PROTOCEREBRUM OF MICROPTERYX.